XIII. — *On the Estimation of Sugar in Diabetic Urine by the loss of Density after Fermentation.*

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When saccharine urine is fermented with yeast its specific gravity, previously ranging from 1030 to 1050, falls to 1009 or 1002, or even below 1000. This result is chiefly due to the destruction of the sugar it contained, but partly also to the generation of alcohol, and its presence in the fermented product.

As the diminution of density must be proportional to the quantity of sugar broken up by the ferment, the amount of this diminution evidently supplies a means of calculating how much sugar any urine contains, always provided that the remaining ingredients of the urine continue unchanged, or become changed in some uniform ratio.

In order to ascertain the exact relation subsisting between the density lost on fermentation and the sugar destroyed, experiments were made on the fresh urine of several diabetic patients in the Royal Infirmary.

The following procedure was adopted:

1. The amount of sugar per 100 parts was first accurately determined by the volumetrical method, with Fehling's test solution.

2. Next the density was taken by the specific gravity bottle.

3. About 4 *oz.* of the urine were then placed in a 12 *oz.* bottle, with a drachm or two of German yeast, and set aside in a warm place to ferment, taking care to cover the mouth of the bottle with a slip of glass or a loose cork.

4. In from twelve to eighteen hours fermentation was usually over, and at the end of twenty-four hours the froth and scum had subsided or been dissipated sufficiently to permit the density to be again taken.

Operating in this way on a urine passed on the 21*st* of April, I obtained the following results:

Sugar per 100 parts by the volumetrical method ...	7·69
Density before fermentation at 60° or D.....	1038·60
Density after fermentation at 60° or D′.....	1005·92
Density lost or D − D′	32·68

The relation, therefore, between the density lost and the per centage of sugar in this instance was as 32·68 to 7·69, or as 1 to 0.235; so that by multiplying the density lost into the co-efficient 0·235 we have for product the amount of sugar per 100 parts which this urine contained. That is, sugar per 100 parts or $S = (D - D') \times 0{\cdot}235$.

On repeating the experiment a great number of times with different specimens of urine and different specimens of yeast, the number 0·230 was found to be the more exact co-efficient.

The degree of exactitude with which the quantity of sugar may be determined by this method is very great; indeed with the precautions to be mentioned presently it seems susceptible of nearly as much accuracy as the volumetrical method.

The following table places in comparison twenty observations made by the two methods on various diabetic urines, with densities ranging from 1031·52 to 1053·48:

TABLE I.

No.	Sugar per 100 parts by the formula $S=(D-D')\times 0{\cdot}23$.	Sugar per 100 parts by direct volumetrical analysis.	Difference.
1	7·51	7·69	0·18
*2	7·47	7·69	0·22
3	6·68	6·66	0·02
*4	6·72	6·66	0·06
5	5·16	5·18	0·02
*6	5·19	5·18	0·01
7	5·65	5·77	0·12
*8	5·65	5·77	0·12
9	4·47	4·35	0·12
*10	4·49	4·35	0·14
11	7·85	8·06	0·21
12	5·91	6·10	0·19
13	11·27	11·36	0·09
*14	11·21	11·36	0·15
15	5·69	5·68	0·01
16	8·11	8·06	0·05
*17	8·09	8·06	0·03
*18	8·00	8·06	0·06
19	8·29	8·20	0·09
20	7·61	7·74	0·13

These results are so close that the discrepancies may be regarded as due to errors of manipulation rather than to any fault in the method.

In pursuing the inquiry further it was found that the volumetrical analysis, in spite of every care in its performance, did not possess the delicacy and certainty required in a standard when minute differences were concerned; insomuch that, when a discrepancy appeared between the indications of the volumetrical and the fermentation methods, it was found impossible to decide in which proceeding the error lay.

In order therefore still further to test the constancy of the results, artificial diabetic urines were prepared by diluting a natural diabetic urine with known volumes of water, or of a healthy non-saccharine urine. Assuming the estimate of the sugar in the original urine to be correct, the quantity of sugar in the dilutions could be ascertained

* Those marked with an asterisk are duplicate experiments.

with almost absolute accuracy by a simple calculation. In the following table the results obtained by fermenting these dilutions are placed side by side with the calculated quantity of sugar.

TABLE II.

No.		Sugar per 100 parts according to the formula $S=(D-D')\times 0{\cdot}23$.	Sugar per 100 parts by calculation from the first estimate.	Difference.
1	A natural diabetic urine	5·91	—	—
2	The same mixed with $\frac{1}{10}$ of its bulk of water	5·31	5·32	0·01
3	The same mixed with $\frac{2}{10}$ of its bulk of water	4·71	4·73	0·02
4	The same mixed with $\frac{3}{10}$ of its bulk of water	4·16	4·14	0·02
5	The same mixed with $\frac{1}{10}$ of its bulk of healthy urine	5·34	5·32	0·02
6	The same mixed with $\frac{2}{10}$ of its bulk of healthy urine	4·77	4·73	0·04
7	The same mixed with $\frac{3}{10}$ of its bulk of healthy urine	4·15	4·14	0·01
8	The same mixed with $\frac{9}{10}$ of its bulk of healthy urine	0·70	0·59	0·11

Numbers so nearly alike as those in these two columns may be considered as practically identical. In the last experiment only, where the quantity of sugar was under one per cent., was there a sensible discrepancy.

Satisfied now with the accuracy of the fermentation method, I was desirous of determining with more certainty and exactitude the required co-efficient; which, from the preceding experiments, using the volumetrical analysis as a standard, was fixed at 0·23.

I was unable to obtain grape sugar in sufficient purity to make solutions of known strength, and had recourse to cane sugar; using for the purpose the best loaf sugar of the shops.

Solutions were made, varying in strength from 2 to 10 per cent. both with distilled water and healthy urine. But as cane sugar becomes converted into grape sugar under

the influence of yeast before fermentation begins, and the density of the solution is thereby materially increased, corrections had to be made on both these accounts before the experiments could be fairly compared with those on diabetic urine. In making the first correction cane sugar was taken as C_{12} H_{11} O_{11} and grape sugar as C_{12} H_{12} O_{12}. In making the second correction allowance was made for the increase of density in accordance with the tables published by the authors of the report on "Original Gravities." *

Six solutions were made of cane sugar in water; two containing 10 per cent. and the remaining four containing respectively 8, 6, 4 and 2 per cent. of sugar.

The mean co-efficient obtained from these six experiments was 0·234.

Twelve solutions were similarly made in a healthy non-saccharine urine; two containing 10 per cent., three 8 per cent., and two 2 per cent. The remainder contained respectively 6, 4, 1·4, 1 and 0·6 per cent. These yielded a mean co-efficient of 0·228; and the general mean for the eighteen trials was a fraction under 0·23. These experiments therefore confirmed the results previously obtained with the volumetrical analysis as a standard.

Equally correct results were obtained in operating on weak solutions, containing only 1 or 0·6 per cent. of sugar, where the density lost was not more than 3 or 4 degrees, as with solutions containing 10 per cent. of sugar, in which the loss of density exceeded 43 degrees.

Having examined the question experimentally, and fixed the co-efficient from multiplied trials under varied conditions, it was not without interest to examine a little more closely the several items which go to constitute the "density lost" in fermentation, and to endeavour to arrive

* Report on Original Gravities, by Professors Graham, Hofmann and Redwood, in the *Quarterly Journal of the Chemical Society*, 1853.

synthetically at similar results to those obtained by the more empirical method of direct experiment.

If we take a solution of cane sugar in water, made so that 100 grains are contained in each 1000 grain-measures, its density at 60° will be 1038·64.

Such a solution consists by weight of 100 grains of sugar, and 938·64 grains of water.

After fermentation there remains 53·74 grains of alcohol, and 987·18 grains of water.

Such a mixture of alcohol and water has, according to Gilpin's tables,* a density of 993.

This figure is very near that obtained in actual fermentation. The density of the fermented product is, however, a little higher, about 1½ or 2 degrees. This slight excess is to be attributed to the escape of a portion of alcohol with the carbonic acid during fermentation, and to a little soluble matter taken up from the yeast.

Some effect probably, though I know not of what kind, is likewise produced by the retention in the liquor of more than its own bulk of gaseous carbonic acid in a state of solution.

It was uniformly found, with solutions of equal strength, that those made with urine showed rather more "density lost" than those made with water. This is an indication either that changes take place in some of the non-saccharine constituents of the urine during fermentation — but changes so slight and so constant in their nature that they do not interfere materially with the accuracy of the mode here proposed for estimating sugar — or else that urines usually reputed non-saccharine do in reality contain a small quantity of sugar.

An excessive quantity of yeast was used in these experiments in order to hasten the process of fermentation. The quantity of soluble matter taken up from the yeast was

* See Henry's *Chemistry*, tenth edition, vol. ii. p. 343.

very small. Two drachms shaken up with 4 *oz.* of water, and left in a warm place for thirty-six hours, only increased the density of the water by 0·3°. In the above experiments a piece of yeast about the size of a large filbert or small walnut was employed to ferment four ounces of urine, a little more or a little less making scarcely a sensible difference in the results.

The fermented urine continues somewhat turbid for a day or two after fermentation is completed, and the degree of this turbidity has an appreciable though slight effect on the density. In the experiments detailed in this paper the second density was taken about twenty-four hours after the addition of yeast, and before the urine had completely cleared. It was found in four trials that if the urine was allowed to rest twelve hours longer the density fell by 0·2° or 0·3°. It is desirable, therefore, where a series of experiments is made, and scrupulous accuracy and uniformity are required, always to take the second density at about the same period after fermentation has ceased.

The method here proposed for determining sugar is not put forward in rivalry with the accurate and elegant volumetrical process now usually employed by chemists; but it is believed that it will be of great service to the medical practitioner, who is unaccustomed to delicate chemical manipulations. Any one who has had much practice in the volumetrical method knows that great nicety and considerable experience are requisite to insure trustworthy results. Fehling's test solution also is liable to speedy deterioration unless hermetically sealed from the air. This arises from the conversion of a portion of the tartaric acid of the test into racemic acid, which, equally with sugar, has a reducing power on the oxide of copper, and when present of course vitiates the results. For these reasons it may be surely predicted that the volumetrical method can never come into general use at the bed-side.

The fermentation method, on the other hand, is of exceedingly easy performance, and the taking of densities is an operation to which the medical practitioner is daily accustomed. I shall have an opportunity through another channel to bring this method under the notice of my medical brethren, and to enter more fully on the particulars which concern its clinical application. I content myself here with a short sketch of the process, as it may be conveniently carried out in private or hospital practice.

1. The specific gravity of the urine is taken at the ordinary temperature of the ward or bed-room.

2. Three or four ounces of the urine are poured into a 12 *oz.* phial, together with a lump of German yeast of the size of a large filbert.

3. The bottle is lightly corked, or covered with a slip of glass, and set aside in a warm place to ferment.

4. In about eighteen hours, when the fermentation has entirely ceased, the bottle is tightly corked and removed to the ward or bed-room so that it may cool to the temperature at which the specific gravity was taken the day before.

5. The urine in the meantime clears, and in five or six hours it may be decanted into an appropriate vessel and the specific gravity taken again.

6. The amount of "density lost" is thus ascertained, and the following simple and most convenient rule expresses the result of the analysis. *Each degree of "density lost" indicates one grain of sugar per fluid ounce of urine.* So that in the example already quoted in the earlier part of this paper, where the urine lost 32·68° of density after fermentation, the quantity of sugar indicated was 32·68 grains per ounce, or 653·6 grains per imperial pint.

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